ANALYSIS OF THE EXTENT OF SULFUR REMOVAL AND THE EFFECT ON REMAINING SULFUR

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INTRODUCTION

Hydrodesulfurization (HDS) is used to remove organic sulfur from petroleum oils in the refining process. DBTs bearing alkyl substitutions adjacent to the sulfur atom (referred to as sterically hindered compounds), are the most resistant to HDS, and represent a significant barrier to reaching very low sulfur levels in fuels[1]. Bacteria have been isolated which utilize an oxidative pathway to selectively desulfurize a variety of organic sulfur compounds found in petroleum oils[2]. The molecular mechanisms of dibenzothiophene (DBT) desulfurization by this pathway have recently been described[3]. Previous experiments with *Rhodococcus* sp. ECRD-1 (ATCC 55309) using DBT have shown that it is converted to the hydroxylated sulfur-free end product 2-phenylphenol via an analogous pathway[4]. Corresponding conversions of the sterically hindered compounds 4,6-diethyl DBT, 4,6-dimethyl DBT and 4-ethyl DBT were also demonstrated.

This study evaluates the ability of ECRD-1 to desulfurize feeds encountered in refineries and examines the fate of sulfur remaining in the oil. A middle distillate oil (232 - 343°C) and a vacuum gas oil (VGO) (343 - 496°C), representing a diesel range oil and a heavy gas oil, were tested as sole sulfur sources in batch cultures. Sulfur removal was quantified using the ratio of Flame Ionization (FID) and Sulfur Chemiluminesce (SCD) detector response factors in Gas Chromatography analysis. Results demonstrated that up to 40% of sulfur in the middle distillate cut could be removed in two week batch cultures. Compounds across the entire boiling range of the oil were affected by the treatment. Less than half that removal is evident in the heavier VGO, suggesting limitations on the range of compounds susceptible to desulfurization by this system. Analysis of the chemical state of the sulfur remaining in the treated oils by sulfur K-edge X-ray absorption spectroscopy showed that in the case of the middle distillate oil over 50% of the remaining sulfur was in an oxidized form. A lesser amount of the remaining sulfur in the treated VGO was in an oxidized state, consistent with the degree of desulfurization. The presence of partially oxidized sulfur compounds in the treated oils indicates that these compounds were en route toward desulfurization. Overall, in the case of the middle distillate oil, more than two-thirds of the initial sulfur had been affected by the microbial treatment.

EXPERIMENTAL

Bacterial Strain. *Rhodococcus* sp. ECRD-1 (ATCC 55309), previously designated Arthrobacter sp. D-1 (ATCC 55309), was isolated by enrichment culture from marine sediments based on its ability to selectively remove sulfur from the sterically hindered organic sulfur compound 4,6-DEDBT[1] using the organic sulfur compound as a sole sulfur source.

Media. Mineral Salts Sulfur-Free Medium (MSSF) containing 1% sodium acetate was prepared as previously described[1] and used for oil desulfurization experiments. Tungsten was added as 50 ug/ml $Na_2WO_4 \cdot 2H_2O$ in VGO cultures. Sulfate control media contained 0.2 g $MgSO_4 \cdot 7H_2O$ per liter.

Luria broth (LB) was used to grow cultures for use as and contained per liter: 10 g Difco tryptone, 5 g Difco yeast extract, 5 g NaCl, adjusted to pH 7.0 with 1.0 ml 1 M NaOH and autoclaved at 121°C, 15 psi.

Oils. Oregon Basin (OB) crude oil, a 450 - 650°F (232 - 343°C) middle distillate cut, represents a diesel range oil fraction. The OB oil used for experiments was artificially weathered under a stream of nitrogen to a constant weight to eliminate inconsistencies caused by evaporative loss of oil during culturing or extraction. Weathering resulted in a weight loss of less than 10% and no change in ppm sulfur content. Vacuum gas oil (VGO), a 650 - 950°F (343 - 496°C) distillate cut, represents a heavy oil fraction. The oil used was also artificially weathered under a stream of nitrogen to a constant weight to eliminate inconsistencies caused by

untreated oils was determined by X-ray fluorescence sulfur analysis (XRF) and GC/FID/SCD. The percent sulfur for the Oregon Basin cut sterile control was 2.07% determined by XRF and 1.96 % by GC/FID/SCD. The percent sulfur for the VGO cut sterile control was 2.93% determined by XRF and 2.26% by GC/FID/SCD.

Biodesulfurization Assays. Biodesulfurization were performed as growing cell assays. One ml, approximately 0.84 g, of artificially weathered and heat sterilized oil was treated in one liter of culture. Inocula were prepared from overnight cultures grown from single bacterial colonies in LB at room temperature (23°C) on a VWR Orbital shaker at 200 rpm. Cells were then pelleted in a Sorval centrifuge at 3000 x g in SS34 rotor at 4°C. The pellet was washed three times with one volume of 12 mM phosphate buffer (pH 7.0) previously chilled on ice for 30 min. Cell pellets were resuspended in 1/10 the original culture volume of chilled phosphate buffer and used immediately for inoculation. Flasks were inoculated with 2 ml of the cell suspension per L medium.

Duplicate cultures were incubated with shaking at 200 rpm for 4 days for OB oils. The experiment also included a uninoculated negative control. VGO oils were incubated with shaking at 200 rpm for 5 and 7 days. The five day experiment included a positive control culture, inoculated and containing sulfate. The seven day experiment included a uninoculated negative control. Flasks were pH monitored at one to two day intervals and adjusted to pH 7.0 with 1M phosphoric acid when the pH deviated by more than 1.0 pH unit. All assays were performed in duplicate.

Before extraction, cultures were brought to a pH of 1.0 with 1N HCl. A 0.5 ml aliquot of 1% v/v dodecane in methylene chloride solution was added as an extraction standard. Each flask was then extracted 3X with 100 ml methylene chloride . The methylene chloride extracts were filtered through anhydrous sodium sulfate or calcium sulfate if water was apparent (i.e., a turbid solution was observed). The samples were then reduced to approximately 5 ml volumes under nitrogen. Samples were subsequently filtered through a 0.22 µm hydrophobic filter to remove turbidity (attributed to water condensate) appearing after volume reduction. For every liter of culture extracted, a 0.5 ml aliquot of decane/methylene chloride (0.742 g/100 ml) was added to the filtered extracts as an injection volume standard. The solutions were then concentrated to approximately 1.0 ml.

GC/FID/SCD Analysis. Gas Chromatography was performed on a Perkin-Elmer GC Autosystem (split/splitless injector). Oregon Basin oil was chromatographed on a Supelco SPB-1 column (30m x 0.32mm, 0.25um film thickness). The temperature zones for the GC were as follows: injector and detector temperature 300°C, initial oven temperature 40°C for 1 minute, followed by a 4°C /minute temperature ramp to 300°C for a final 10 minute hold. VGO was chromatographed on a Supelco SPB-1 column (15 m x 0.32mm, 4um film thickness). The temperature zones for the GC were as follows: injector temperature 275°C, detector temperature 325°C, initial oven temperature 50°C for 1 minute, followed by a 5°C /minute temperature ramp to 300°C for a final 20 minute hold. Tandem Perkin-Elmer FID (flame ionization detection) and Sievers Instruments, model 355 SCD (sulfur chemiluminescence detection) detectors were used to determine sulfur concentrations in oil samples based on response factors of model compounds. For oils and standard mixtures, 1 µl of sample was injected in duplicate and results averaged.

Response factors for OB oil and VGO were estimated based on the averaged FID and SCD response factors for a number of model compounds. These compounds were chosen to represent some of the compounds found in the oils. The standards used for calibration of the FID detector were hexane, heptane, decane, dodecane, tetradecane, fluorene, carbazole, DBT sulfone, and 4,6 DEDBT. The sulfur compounds contained in this mixture were used to calibrate the SCD. Averaged response factors for sulfur (ng/area) and for carbon (mg/area) were calculated for the standard mixtures and the sulfur/carbon ratio calculated. This ratio was multiplied by the sulfur/carbon area of the oils to give ppm S. A common baseline was drawn by the computer encompassing all area associated with the oil so that unresolved area characterized by a hump in the baseline was included in subsequent calculations.

The percent carbon loss for treated oils was determined as the difference between the GC/FID area ratio of total carbon (minus standards) to dodecane extraction standard of control and treated samples.

Sulfur K-edge X-ray absorption-edge spectroscopy. Sulfur K-edge X-ray absorption-edge spectroscopy was used to determine the effect of biodesulfurization on the remaining sulfur content of treated oils. This technique allows for the evaluation of the chemical state of sulfur

Stanford Synchrotron Radiation Laboratory. Reference compounds were run as powder films using electron yield detection, while oil samples were run as liquids using fluorescence detection[2]. Spectra of the oils were fitted to linear combinations of the spectra of reference compounds using least-squares non-linear optimization[5]. In general there is a trend toward higher absorbance energies in the order sulfidic, thiophenic, oxidized species (Fig. 1).

The procedure employed to fit the oil spectra employed a fairly broad range of model organic sulfur compounds as reference compounds to represent the majority of sulfur types expected in treated and untreated oils. Fig. 1 shows the sulfur K-edge X-ray absorption spectra of these compounds. Although the individual spectra are quite distinct, different fit results were obtained with good fits using different constraints on the fit calculations. Consequently, the fits obtained using this method are used as indicators of the likely distribution of S compounds and are not considered highly accurate for specific species.

RESULTS AND DISCUSSION

Oregon Basin Oil. The desulfurization of OB oil ECRD-1 cultures grown for four days resulted in a large reduction in sulfur containing compounds. The GC/SCD chromatograms for a sterile control and ECRD-1 desulfurized oil show that sulfur components across the entire boiling range of the oil were effected (Fig. 2). The total sulfur removed was 35 (± 30% RSD). Examination of the GC/FID profile (Fig. 3) revealed a reduction in the resolved peaks (n-alkanes) in the treated cultures. Loss of the straight chain hydrocarbons is attributed to degradation by ECRD-1 which is know to degrade these compounds. The loss of carbon in these samples was averaged 26% (± 7% RSD). Taking into account the consumption of carbon, the reduction of sulfur the maximum sulfur removed is calculated to be 58%.

Analysis of the oils by sulfur K-edge X-ray absorption-edge spectroscopy was performed to determine the effect of treatment on the remaining sulfur in the oil. The sulfur spectra of the treated, sterile control and original oils are shown in Fig. 4. The spectra of the weathered OB oil and the sterile control are virtually identical indicating no abiological effects occurred due to the culture conditions used. In contrast, the spectrum of the treated oil is markedly different, showing an increase in absorbance at approximately 2473 and 2477 eV. A shift in absorbance toward higher energies (eV) is characteristic of more oxidized sulfur species, (see Fig. 1), indicating that a significant proportion of the sulfur compounds remaining in the oil contain sulfur in an oxidized form.

Table 1 shows the best fit composition of sulfur forms in the sterile and treated oil. A small feature near 2480 eV was observed for both samples and is attributed to sulfate contaminating the graphite used for sample preparation.

Vacuum Gas Oil. Cultures of ECRD-1 grown on VGO as the sole sulfur source for five and seven days showed growth and sulfur reduction. The inoculated positive control containing sulfate also showed growth but no sulfur removal. The negative controls showed no growth. After incubation the concentration of sulfur in the VGO in the positive control and the sterile control cultures were equivalent, both approximately 2.3%. The lack of desulfurization in the presence of sulfate is consistent with previous observations that sulfate represses the expression of desulfurization activity in ECRD-1 (Table 2). The 7 day ECRD-1 cultures showed a 16% sulfur reduction as compared to only 7% for the five day cultures. The difference in desulfurization levels demonstrates that additional desulfurization of VGO is achieved with extended incubation periods. However, in comparison to OB oil, VGO appears relatively more resistant to desulfurization by ECRD-1.

Analysis of the treated VGO samples by X-ray spectroscopy showed an increase in absorbance at approximately 2477 eV in all treated samples (Fig. 5). This increase in absorbance is consistent with the production of oxidized sulfur compounds, albeit considerably less than that observed with the OB oil. No changes in the spectrum were observed for the culture amended with sulfate (data not shown) corroborating the GC results and demonstrating that no detectable desulfurization activity occurred. Due to the relatively small change in the spectrum for the treated VGO the changes in oxidized species could not be meaningfully fit with model compounds.

CONCLUSIONS

This study has shown that ECRD-1 can desulfurize complex sulfur found in a middle distillate cut and a vacuum gas oil. Consumption of hydrocarbons could impact negatively on process economics. However, there is no indication that hydrocarbon degradation is tied to desulfurization. Hydrocarbon degradation activity could be eliminated through genetic

microbial treatment, complete desulfurization was not achieved. However, a significant percentage of the remaining sulfur is oxidized by the treatment indicating the potential for further desulfurization. Additionally, the ability to remove sterically hindered compounds not affected by HDS could prove valuable.

[1] Kabe, T., Ishihara, A. and Tajima, H. 1992. Ind. Eng. Chem. Res. 31:1577-1580.

[2] Grossman, M.J., in "Hydrotreating technology for pollution control: Catalysts, Catalysis, and Processes" (M. L. Occelli and R. Chianelli, eds.) Marcel Dekker, New York, pp. 345-359, 1996.

[3] Gray, K.A., Pogrebinsky, O.S., Mrachko, G.T., Xi, Lei, Monticello, D.J., and Squires, C.H. 1996. Nature Biotechnology 14:17051709.

[4] Lee, M.K., Senius, J.D., and Grossman, M.J. Appl. Environ. Microbiol. 61:4362-4366 (1995).

[5] George, G.N., Gorbaty, M.L., Kelemen, S.R., and Sansone, M. Energy & Fuels. 5: 93-97 (1991).

TABLE 1
Composition Of Sulfur Species In Treated Oregon Basin Crude Oil
Analysis by Sulfur K-edge X-ray Absorption-edge Spectroscopy

Model compound	Relative % total S as this species	
	Sterile control (2.0% S)	ECRD-1 treated (1.1% S)
2,5 Dimethylthiophene	51	24
Benzyl sulfide	47	18
Dimethylsulfoxide	-	32
Dibenzothiophene sulfone	-	24
Sulfate	2	2

TABLE 2
Sulfur Concentration in VGO Cultures
Analysis by GC/FID/SCD

Sample	% Sulfur(±%RSD)	% S Removed
5 day culture Positive Control	2.27±4%	-
5 day culture ECRD-1 Treated	2.11±0.1%	7.0
7 day culture Negative Control	2.25±1%	-
7 day culture ECRD-1 Treated	1.91±3%	15.5

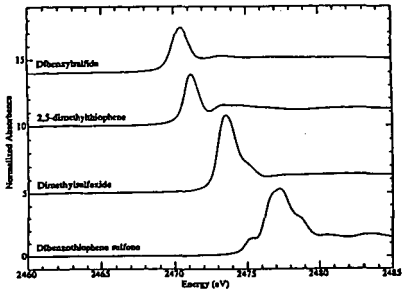

Figure 1. Sulfur K-edge X-ray Absorption Spectra of reference compounds.

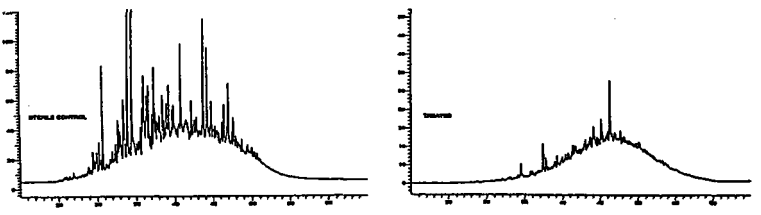

Figure 2. GC/SCD of sterile control and treated Oregon Basin oil.

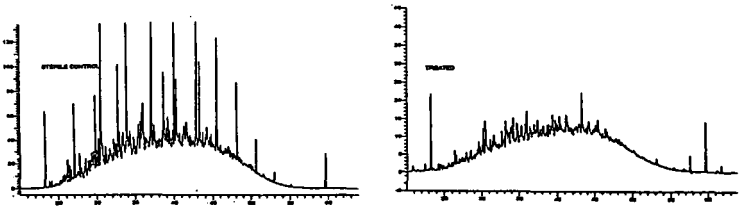

Figure 3. GC/FID of sterile control and treated Oregon Basin oil.

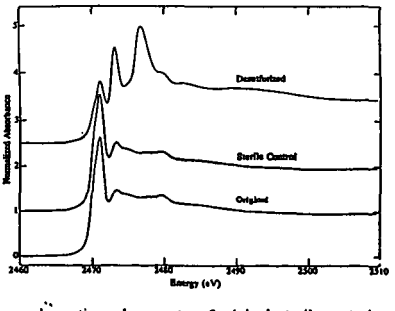

Figure 4. Sulfur K-edge X-ray absorption-edge spectra of original, sterile control and treated Oregon Basin Oil.

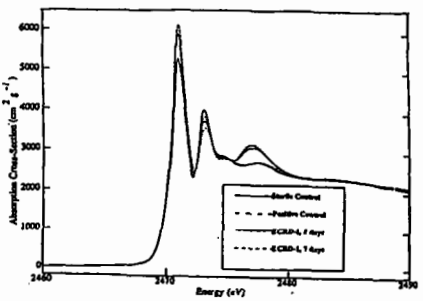

Figure 5. Sulfur K-edge X-ray Absorption spectra of Vacuum Gas Oil sterile control, positive control, and five and seven day ECRD-1 treatment